REMARKS

Claims 25, 28 and 29 are now pending for prosecution in this case. With this Amendment, claim 25 has been amended and new claims 30-37 added. Support for the claim amendment and new claims can be found throughout the specification, *inter alia*, from page 8, lines 9-35 to page 9, lines 1-9; pages 14-15; and in the Examples, for e.g., pages 28-32. The amendments were generally made for clarity. No new matter has been added.

With respect to all amendments, Applicant has not dedicated or abandoned any unclaimed subject matter and moreover has not acquiesced to any objection and/or rejection made by the Office. Applicant expressly reserve the right to pursue prosecution of any presently excluded claimed subject matter or embodiments in one or more future continuation and/or divisional applications.

Attached hereto is a marked-up version of the changes made to the specification and claims by the current amendment. The attached page is captioned "**Version with markings to show changes made.**"

Statement of Related Applications

The following application is related to the present application:

U.S. Application Serial No. 08/199,268, filed February 28, 1994.

Form 1449/Information Disclosure Statement

Applicant thanks the Examiner for attaching to the outstanding Office Action an Examiner-initialed Form PTO-1449 listing references 94-95. However, Applicant has not received an **Examiner-initialed** copy of the Form PTO-1449 that was submitted at the time of filing of the instant application on November 15, 2000. Said From PTO-1449 consisted of 5 sheets, listing references 1-93. A copy of the initialed form is respectfully requested from the Examiner.

Claim Rejections - 35 USC §112, second paragraph

Claim 25 stands rejected because the phrases "entirely free of" and "entirely homogenous" are allegedly not clear with respect to their metes and bounds.

Applicant respectfully traverses.

Applicant respectfully submits that the skilled artisan, in view of the teachings of the specification and the state of the art, would understand the metes and bounds of these phrases, and thus

the scope of claim 25. However, in order to expedite prosecution, Applicant has amended claim 25 by deleting the term "entirely" to address the apparent redundancy in the use of the term in conjunction with "free of" and "homogenous".

In view of the discussion above, Applicant respectfully requests that these rejections under 35 U.S.C. §112, second paragraph, be withdrawn.

Claim Rejections - 35 USC §112, first paragraph

Claim 25 stands rejected under 35 U.S.C. 112, first paragraph as allegedly containing subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to ***make*** the invention.

As an initial matter, Applicant notes that the Examiner's concern is with how to *make* the invention, and that there is not an issue with how to use the claimed invention.

First, the Examiner contends that "neither the specification nor the art teaches how to get homogenous protein molecules, $(Fab')_2$ as to the heavy chain C-terminal amino acid residues, especially when the protein is subjected to many proteolysis-inducing manipulations such as protein purification procedures and other chemical procedures." Office Action, page 3. Second, the Examiner asserts that "neither the specification nor the art teaches how to cross-link two Fab' fragment with multiple SH groups at the hinge region while [sic] preventing formation of intrachain disulfide bond at the hinge region." Office Action, page 3. Applicant addresses these two points in order below.

As a preliminary matter, Applicant respectfully submits that the scope of the claimed invention should be read in view of the teachings of the specification and the state of the art at the time of filing of the application. It would be clear to the skilled artisan from the teachings of the specification and the state of the art that $F(ab')_2$ compositions generated by methods of the invention would be substantially free of $F(ab')_2$ having hinge region intrachain disulfide bonds and substantially homogenous as to the heavy chain C-terminal amino acid residue. The specification provides both general and specific guidance for making (and using) the invention. General guidance is provided throughout the specification. Extensive specific guidance can be found, *inter alia*, in the working examples described in the Examples section (*see* pages 28-35, Table 1, and Figures 1 & 2). The examples illustrate the generation and characterization of compositions that illustratively embody the scope of the claimed invention.

Notwithstanding the discussion above, Applicant respectfully submits that the Examiner has not provided reason to doubt the objective truth of the teachings of the specification, as required under the law of enablement. Applicant addresses the Examiner's specific concerns as follows.

Regarding the first point, Applicant respectfully traverses. Applicant respectfully disagrees that Voet et al., at page 77 (*Biochemistry*, 1990, John Wiley and Sons, Inc.) (as cited by the Examiner), teach that "obtaining homogenous protein molecule is not plausible." Office Action, page 3. While Voet et al. note that cells contain proteases and other degradative enzymes which, upon lysis, are liberated into solution along with the protein of interest, they do not make the assertion as the Examiner contends. At best, Voet et al. merely caution that "[c]are must be taken that the protein is not damaged by these enzymes" (Voet et al., page 77, left column, 5th full paragraph]; but Voet et al. go on to teach that "these enzymes can often be specifically inhibited by chemical agents without affecting the desired protein." Voet et al., *supra*. Consistent with this, the instant specification provides extensive teachings regarding use of protease inhibitors (*see*, for e.g., specification page 15, lines 15-17; page 31 (Examples), lines 7-11). Furthermore, the specification describes purification methods for removing any proteolytic fragments that may be present. *See* for e.g., specification page 15, lines 10-15, and in the Examples page 31, lines 11-13. Thus, both the art and the teachings of the specification support Applicant's teaching of how to make the claimed invention contrary to the Examiner's contention.

Applicant respectfully traverses the second point. Applicant first notes that, contrary to the Examiner's contention, Glennie et al. (which the Examiner cites at page 2368 left column, 3rd paragraph[1] and Figure 8), do not limit their teaching to making $F(ab')_2$ molecules free of hinge region intradisulfide bond by cross-linking rabbit Fab' fragment "which has *only one* SH group at the hinge region". (emphasis added), Office Action, page 3. Nowhere in this cited reference is there an explicit, nor even an implicit, statement relating to the Examiner's assertion. Thus, Applicant respectfully submits that Glennie et al. does not provide the requisite evidence to explain why one skilled in the art would doubt that the specification is enabling of the claimed invention, as required under the law of enablement. This is true even in light of US Pat. 5,274,119, which the Examiner contends teaches at column 16 and 17 (further noting the first page figure) that hinge region Fab-SH can easily form intradisulfide bond. The Examiner alleges that "[a] few molecules of any of Fab'-TNB, maleimidated Fab', or Fab'-SH might have formed hinge region intradisulfide bond." Office Action, page 4. Applicant respectfully submits that the disclosure of US Pat. 5,274,119 is not applicable, and indeed is contrary, to the nature of the claimed

[1] This paragraph refers to a description of antibodies under the "Material And Methods" section, which does not appear to relate at all to the Examiner's contention.

invention. US Pat. 5,274,119 generally throughout, and specifically at columns 16 & 17, relates to methods of preparing Fab'-SH *from* $F(ab')_2$ (*see* column 15, line 48, column 16, line 41) by selective chemical reformation of H-L disulfide bonds or preparation of Fab'-SH by air oxidation. *See*, for e.g., titles of Examples 4 & 5. A major goal of these methods is to *re-form disulfide bonds* between heavy and light chains, while leaving at least one free hinge region sulfhydryl group available for further reaction. One of skill in the art would understand that conditions intended to achieve re-formation of disulfide bonds between heavy and light chains would also be at least somewhat permissive for *intra*chain disulfide bond formation between two heavy chain (e.g., hinge region) sulfhydryl groups. These circumstances are distinguishable from the conditions under which the claimed invention is made, for example, where Fab' and $F(ab')_2$ molecules are made under conditions designed to substantially inhibit, where necessary, formation of hinge region intrachain disulfide bonds. Indeed, the instant specification generally, and specifically at page 15, lines 19-20, and in the Examples at page 31, lines 7-9, provides ample examples of methods and conditions capable of inhibiting disulfide bond formation.

In view of the above, Applicant respectfully submits that there is no basis to doubt that the specification teaches how to make the claimed invention. Therefore, withdrawal of the rejection under 35 U.S.C. §112, first paragraph is respectfully requested.

CONCLUSION

Applicant believes that this application is now in condition for immediate allowance and respectfully requests that the outstanding rejections be withdrawn and this case passed to issue. No new matter has been introduced, and entry of these amendments is respectfully requested. Reconsideration and further examination of the claims is respectfully requested.

The Examiner is invited to contact the undersigned at (650) 225-5530 in order to expedite the resolution of any remaining issues.

Respectfully submitted,
GENENTECH, INC.

Date: February 28, 2003

By: _____
Paul Naik, Ph.D.
Reg. No. 49,075

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PATENT TRADEMARK OFFICE

VERSION WITH MARKINGS TO SHOW CHANGES MADE

In the claims:

Please amend Claim 25 as follows:

25. (Amended) A composition comprising monospecific $F(ab')_2$ which is:

(a) [entirely] free of $F(ab')_2$ having hinge region intrachain disulfide bonds;

(b) [entirely] free of contaminating arsenite; and

(c) [entirely] homogenous as to the heavy chain C-terminal amino acid residue.

Please add new claims 30-37.

30. (New) A composition comprising $F(ab')_2$ produced by:

(A) expressing an immunoglobulin presequence comprising a first Fab' in a microbial host cell culture under conditions suitable for the secretion of said first Fab' to the periplasmic space of the host cell and formation of Fab'-SH, said first Fab' being capable of binding a first epitope;

(B) expressing an immunoglobulin presequence comprising a second Fab' in a microbial host cell culture under conditions suitable for the secretion of said second Fab' to the periplasmic space of the host cell and formation of Fab'-SH, said second Fab' being capable of binding a second epitope;

(C) recovering said first and second Fab'-SH from said host cells; and

(D) forming a covalent bond between a free thiol cysteinyl residue of said first and second Fab'-SH to form bivalent $F(ab')_2$.

31. (New) The composition of claim 30, wherein step (D) comprises forming said covalent bond in vitro.

32. (New) The composition of claim 31, wherein step (D) comprises:

(a) reacting the first Fab'-SH with (i) 5,5'-dithiobis (2-nitrobenzoic acid) (DTNB) to form a thionitrobenzoate derivative Fab'-TNB or (ii) a bifunctional maleimide;

(b) directly coupling said first Fab'-TNB or maleimidated Fab' to the second Fab'-SH to form a $F(ab')_2$; and

(c) recovering said $F(ab')_2$.

33. (New) The composition of claim 30, wherein the $F(ab')_2$ is bispecific.

34. (New) The composition of claim 30, wherein the $F(ab')_2$ is monospecific.

35. (New) The composition of claim 30, wherein each Fab' of the $F(ab')_2$ has only one hinge region cysteine.

36. (New) The composition of claim 30 which is:

(a) substantially free of $F(ab')_2$ having hinge region intrachain disulfide bonds;

(b) substantially free of contaminating arsenite; and

(c) substantially homogenous as to the heavy chain C-terminal amino acid residue.

37. (New) The composition of claim 30, wherein each Fab' comprises the C-terminal amino acid sequence Cys Ala Ala.

CLEAN SET OF PENDING CLAIMS

25. A composition comprising monospecific $F(ab')_2$ which is:

(a) free of $F(ab')_2$ having hinge region intrachain disulfide bonds;

(b) free of contaminating arsenite; and

(c) homogenous as to the heavy chain C-terminal amino acid residue.

28. The composition of claim 25 wherein each Fab' of the $F(ab')_2$ has only one hinge region cysteine.

29. The composition of claim 28 wherein each Fab' comprises the C-terminal amino acid sequence Cys Ala Ala.

30. A composition comprising $F(ab')_2$ produced by:

(A) expressing an immunoglobulin presequence comprising a first Fab' in a microbial host cell culture under conditions suitable for the secretion of said first Fab' to the periplasmic space of the host cell and formation of Fab'-SH, said first Fab' being capable of binding a first epitope;

(B) expressing an immunoglobulin presequence comprising a second Fab' in a microbial host cell culture under conditions suitable for the secretion of said second Fab' to the periplasmic space of the host cell and formation of Fab'-SH, said second Fab' being capable of binding a second epitope;

(C) recovering said first and second Fab'-SH from said host cells; and

(D) forming a covalent bond between a free thiol cysteinyl residue of said first and second Fab'-SH to form bivalent $F(ab')_2$.

31. The composition of claim 30, wherein step (D) comprises forming said covalent bond in vitro.

32. The composition of claim 31, wherein step (D) comprises:

(a) reacting the first Fab'-SH with (i) 5,5'-dithiobis (2-nitrobenzoic acid) (DTNB) to form a thionitrobenzoate derivative Fab'-TNB or (ii) a bifunctional maleimide;

(b) directly coupling said first Fab'-TNB or maleimidated Fab' to the second Fab'-SH to form a $F(ab')_2$; and

(c) recovering said $F(ab')_2$.

33. The composition of claim 30, wherein the $F(ab')_2$ is bispecific.

34. The composition of claim 30, wherein the $F(ab')_2$ is monospecific.

35. The composition of claim 30, wherein each Fab' of the $F(ab')_2$ has only one hinge region cysteine.

36. The composition of claim 30 which is:

(a) substantially free of $F(ab')_2$ having hinge region intrachain disulfide bonds;

(b) substantially free of contaminating arsenite; and

(c) substantially homogenous as to the heavy chain C-terminal amino acid residue.

37. The composition of claim 30, wherein each Fab' comprises the C-terminal amino acid sequence Cys Ala Ala.